# THE SEARCH STRUCTURE OF TERPINEN-4-OL USING ITS FRAGMENTATION ENERGY PROFILE: CASE STUDY FOR A GC-MS ANALYSIS OF THE LAVENDER OIL

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*Abstract: The automated elucidation by GC-MS of isomeric structures by using mass spectral libraries and retention indexes databases often offers contradictory results in the case of the high similarity of the structures and the spectra. Under these circumstances, a certification of the unknown structures by using additional orthogonal filtering of candidate structures would increase the confidence in the analysis. For such additional filtering we propose here a correlation algorithm between the profile of some primary ionic currents and that of corresponding fragmentation energies obtained through quantum chemical calculation (QCC). In our paper we present the application of this algorithm in the case of terpinen-4-ol, a biologically active compound from essential oils. The fragmentation energy profile of the terpinen-4-ol has been identified with the maximum correlation (94.2%) among the fifteen components revealed in GC-MS analysis of lavender oil. An additional validation was done by applying the algorithm for another fourteen candidate structures, isomeric with terpinen-4-ol. The result is consistent with the library-based assignments and chromatographic alignment. One of the advantages of this approach is that the use of these three analytical variants can provide high accuracy of analysis without the direct use of chemical standards.*

**Keywords**: differential mass spectrometry, structure identification, fragmentation energy, lavender oil, terpinen-4-ol

## INTRODUCTION

The limitations of mass spectrometry regarding the automated identification of isomeric structures with similar mass spectra are well known. In these cases, the discrimination between isomers through the interpretation of fragmentation patterns is not feasible, because the mass spectra of these isomeric analytes contain the same signals (Kind et al. 2007). The spectral similarity of a great number of essential oils causes difficulty in obtaining positive identification of individual components. The mass spectrum library-search inevitably offers a range of false positive responses. A post-search filter using experimental or calculated retention index (RI) rejects candidate structures whose retention index is not consistent with the retention index of the unknown component (Shellie et al. 2002). However, it should be taken into account that the agglomeration of RI flavour and fragrance compounds (FFC), its confidence interval (E.g., ±5 index units) and the measurement and calculation errors of RI can force a false positive result, especially when there are several similar candidate structures. In this case, a certification of the unknown structure by an additional orthogonal filtering would increase the confidence in the analysis. For this purpose, we used an algorithm based on differential mass spectrometry ($\Delta$MS) quantitative and structure-fragmentation relationship (QSFR) strategy (Dinca et al. 2012). This uses the inverted linear correlation (LCOR) of the intensities' series of the main isobaric ions with the corresponding calculated enthalpies' series (Dinca et al. 2014). Because no unified enthalpies databases exist, these were calculated using quantum chemical methods, usually semi-empirical methods, which give reliable values and which can be quickly implemented on ordinary computers (Rocha et al. 2006). In this paper we propose to validate this algorithm for the identification of terpinen-4-ol, a biologically active compound from lavender oils.

**MATERIALS AND METHODS**
The preparation of the lavender essential oil and its GC-MS analysis were presented in previous papers (Copolovici et al. 2017). The ionization energy was 20 and 70 eV. The GC-MS chromatogram of lavender oil at 20 eV is shown in Figure 1.

*The strategy of $\Delta_f H$ database calculation.* The heats of formation ($\Delta_f H$) were calculated with the semi-empirical method RM1 using the HyperChem 8.0.10 software. The geometries of the molecules and radicals were optimized with the MM+ force field and re-optimized, using the RHF operators for molecules or ions and UHF for radicals (Rocha et al. 2006), the molecule being considered in vacuum. The fragmentation enthalpies ($\Delta_f H$(frag)) were calculated according to eqn.1:

$$\Delta_f H(\text{frag}) = \Delta_f H(\text{ion}) + \Sigma\Delta_f H(\text{F}) - \Delta_f H(\text{M}) \quad (1)$$

where $\Delta_f H$(ion) is the formation enthalpy of the main ion, $\Sigma\Delta_f H$(F) is the sum of the formation enthalpies of secondary fragments (radicals and molecules) and $\Delta_f H$(M) the formation enthalpy of the molecule (Dinca et al. 2014).

**RESULTS AND DISCUSSIONS**
The chromatographic integration revealed fifteen chemical entities in lavender oil (Figure 1). The chromatographic alignment and library-based assignments indicates the terpinen-4-ol as the ninth compound of the chromatogram with a retention time of 17.91 minutes.
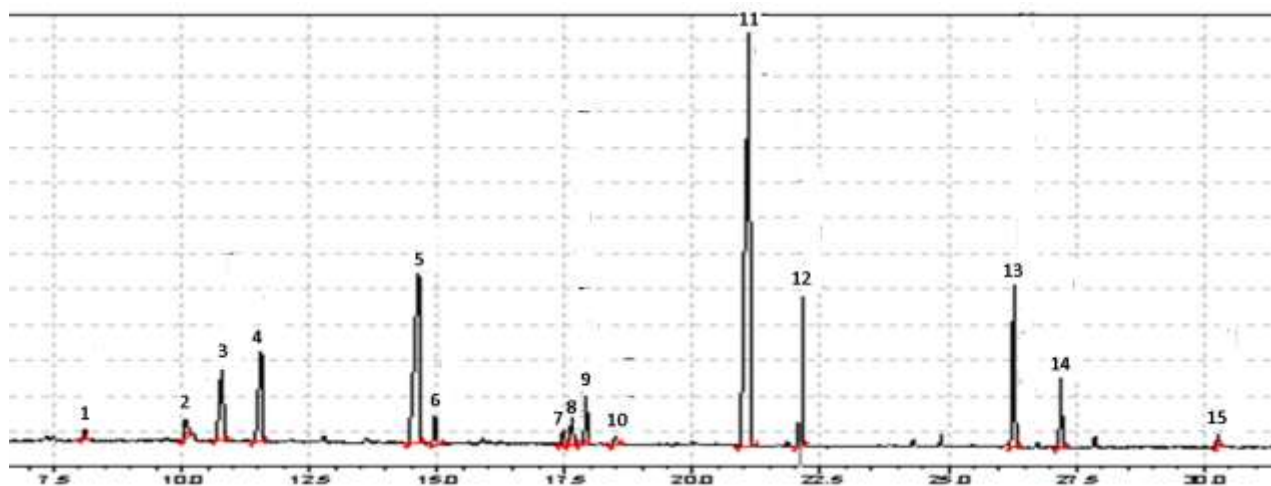


**Figure 1.** The GC-MS chromatogram of the lavender oil.

To increase the confidence of the analysis, terpinen-4-ol was also searched for among the fifteen components using a QSFR strategy based on its energy profile. It contains the molecular ionization / fragmentation energies for five principal ions: $M^{+\cdot}$ at m/z 154, M-CH$_3$]$^+$ at m/z 139, M-OH]$^+$ at m/z 137, M-H$_2$O]$^{+\cdot}$ at m/z 136 and M-H$_2$O-CH$_3$]$^+$ at m/z 121 (Table 1).

**Table 1** The fragmentation energies of terpinen-4-ol

|  | m/z 154 | m/z 139 | m/z 137 | m/z 136 | m/z 121 |
|---|---|---|---|---|---|
|  | $\Delta_f H$ frag (kcal/mol) | | | | |
| Terpinen-4-ol | 189.3 | 244.0 | 244.4 | 184.2 | 224.3 |

The corresponding ionic currents (ICs) were collected at the maximum of each chromatographic peak (Table 2). The linear correlation of calculated energy profile of

terpinen-4-ol with each of the fifteen ICs series leads to coefficients *R* that are converted into probabilities using eqn. 2:

$$P_{\text{LCOR(\%)}} = 100(1 - R)/2 \qquad (2)$$

The resulted probabilities are presented in the last column of Table 2. The maximum probability (94.2%) corresponds to the ninth GC peak. In this way, the presence of terpinen-4-ol has also been confirmed in the lavender oil.

**Table 2.** The series of ICs (arbitrary units) for the five principal ions used for energetic profile. The correlation probability (*P*%) with energy profile of terpinen-4-ol is maximal for the ninth GC peak.

| GC peak | **Ionic currents** | | | | | |
|---|---|---|---|---|---|---|
| | m/z 154 | m/z 139 | m/z 137 | m/z 136 | m/z 121 | *P*(%) |
| 1 | 501 | 522 | 752 | 3004 | 3899 | 64 |
| 2 | 14844 | 25410 | 1989 | 6309 | 7173 | 42 |
| 3 | 524 | 494 | 653 | 2255 | 26097 | 45 |
| 4 | 494 | 486 | 844 | 6079 | 48681 | 47 |
| 5 | 1436 | 7519 | 4742 | 22077 | 126705 | 46 |
| 6 | 486 | 592 | 500 | 484 | 544 | 16 |
| 7 | 90 | 3566 | 428 | 3037 | 6765 | 42 |
| 8 | 31 | 413 | 313 | 1200 | 7921 | 45 |
| **9** | **11604** | **4604** | **2538** | **23814** | **7276** | **94.2** |
| 10 | 10 | 295 | 657 | 8944 | 14403 | 61 |
| 11 | 15890 | 13644 | 12528 | 70634 | 452033 | 47 |
| 12 | 2916 | 1156 | 5232 | 47895 | 161007 | 52 |
| 13 | 514 | 496 | 3116 | 8879 | 72012 | 46 |
| 14 | 486 | 499 | 1496 | 1548 | 14850 | 43 |
| 15 | 506 | 575 | 1850 | 1496 | 5589 | 41 |

The accuracy of the algorithm was verified in the case of some stereoisomers and positional isomers of terpinen-4-ol with similar mass spectra and close RI. Their structures are shown in Figure 2: borneol (**1**), iso-borneol (**2**), terpinen-4-ol (**3**), α-terpineol (**4**), 1-myrcenol (**5**), bicyclo[2.2.1]heptan-1-ol, 4,7,7-trimethyl- (**6**), bicyclo[2.2.1]heptan-2-ol, 4,7,7-trimethyl-, (1*S-exo*)- (**7**), bicyclo[2.2.1]heptan-2-ol, 4,7,7-trimethyl-, (1*S-endo*)- (**8**), linalool (**9**), nerol (**10**), geraniol (**11**), terpinen-1-ol (**12**), β-

terpineol (**13**), δ-terpineol (**14**), γ-terpineol (**15**). The filtration based on energy profile establishes that none of this candidate structures get a higher probability than terpinen-4-ol (Table 3).
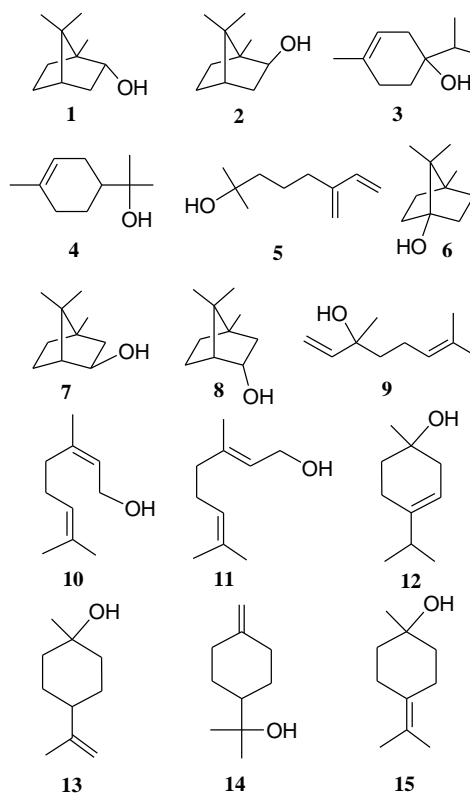


**Figure 2.** Stereoisomers and positional isomers of terpinen-4-ol, possible candidate structures for the ninth GC peak.

**Table 3**. The $\Delta_f H$ frag profiles of the fifteen candidate structures. The correlation probability (*P*(%)) of this profiles with ICs series of the ninth GC peak is maximum for structure **3** (terpinen-4-ol).

| Structure | $\Delta_f H$ frag (kcal/mol) | | | | | |
|---|---|---|---|---|---|---|
| | m/z 154 | m/z 139 | m/z 137 | m/z 136 | m/z 121 | *P*(%) |
| 1 | 198.5 | 239.9 | 255.3 | 216.1 | 232.2 | 83 |
| 2 | 205.6 | 234.9 | 255.0 | 215.8 | 231.9 | 85 |
| **3** | **189.3** | **244.0** | **244.4** | **184.2** | **224.3** | **94.2** |
| 4 | 190.0 | 221.7 | 248.1 | 196.6 | 265.3 | 83 |
| 5 | 193.1 | 223.7 | 247.6 | 203.9 | 271.0 | 79 |
| 6 | 206.2 | 239.5 | 280.2 | 247.3 | 287.9 | 67 |
| 7 | 204.9 | 235.5 | 256.2 | 216.6 | 232.7 | 85 |
| 8 | 203.8 | 239.3 | 255.9 | 216.3 | 232.4 | 85 |
| 9 | 191.3 | 216.8 | 239.1 | 192.6 | 262.5 | 82 |

| | | | | | | |
|----|-------|-------|-------|-------|-------|----|
| **10** | 194.3 | 252.0 | 240.5 | 212.8 | 262.7 | 80 |
| **11** | 191.6 | 257.9 | 242.8 | 215.3 | 265.2 | 79 |
| **12** | 187.5 | 218.8 | 243.9 | 183.2 | 222.4 | 93 |
| **13** | 203.6 | 219.5 | 244.1 | 197.5 | 263.9 | 84 |
| **14** | 198.7 | 221.7 | 248.1 | 202.6 | 263.1 | 82 |
| **15** | 189.6 | 219.5 | 245.6 | 199.4 | 267.5 | 80 |

The algorithm offers good sensitivity and selectivity by distributing structures **1**-**15** in a probability range of nearly 30 units. The proportionality between structural and probability differences is due to the ability of QCC to provide unitary formation enthalpies which can includes the finest structural details. E.g., the position isomers **3** and **12** are separated in the list by $\Delta P$=1.2%, while structure **3** compared to the chain isomers **9**, **10** and **11**, by $\Delta P \approx 15$%. The calculated energy profiles places the terpinen-4-ol structure on the first place in the list of probabilities, just like library-based assignments and chromatographic alignment.

## CONCLUSIONS

At least for the primary ions of terpinen-4-ol isomers, the inverse proportionality between ionic currents and fragmentation energies can be evaluated by GC-MS and QCC and used in analytic purpose. There are wide prospects for generalizing, optimizing and automating this technique because it has important advantages: do not require chemical standards, uses common PC equipment and software, is orthogonal with the currently applied analytical methods and can establish integral *de novo* structures.

## ACKNOWLEDGEMENTS

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